AMENDMENTS TO THE SPECIFICATION

Replace the paragraph at page 6, lines 18-22 with the following paragraph:

"Fig. 5 is a bar graph showing the "long-term"response of synchronized DLD1-neo and DLD1-chfr cells exposed transiently to mitotic stress, e.g., to nocodazole (Noc) or ~~taxol~~ the TAXOL™ drug (T) 12 hours after release from the G1-S block for a 4 hour period. The cells were replated and scored for colony formation 3 weeks later. The controls are indicated by (-)."

Replace the paragraphs at page 6, line 29 through page 7, line 15 with the following paragraphs:

"Fig. 7B is a bar graph showing mitotic index of unsynchronized SAOS2 cells transiently-transfected with plasmids expressing no Chfr protein (vec), wild-type Chfr or ChfrΔFHA. ~~Taxol~~ The TAXOL™ drug was added 36 hours after the transient transfection and the mitotic index was determined 8 (white bar), 12 (gray bar), 14 (first black bar) and 16 (second black bar) hours later.

Fig. 7C is a bar graph illustrating the mitotic index of unsynchronized DLD 1 cells transiently-transfected with plasmids expressing no Chfr protein (DLD1-vec), wild-type Chfr (DLD-chfr; 1 μg), $ChfrMet_{580}$ (DLD1-M_{580}; 5 μg), ChfrΔFHA (DLD1-ΔFHA; 5 μg), or wild-type Chfr (1 μg) and $ChfrM_{580}$ (5 μg) (DLD1-chfr+M_{580}), or wild-type Chfr (1 μg) and ChfrΔFHA (5 μg) (DLD1-chfr+ΔFHA). ~~Taxol~~ The TAXOL™ drug was added 36 hours after the transient transfection and the mitotic index was determined 16 hours later.

Fig. 8A is a graph showing mitotic index of synchronized DLD1 cells stably-transfected with plasmids expressing neo (DLD1-neo) as a function of time in hours after release from the G1-S block. The cells were either not exposed to mitotic stress (□) or treated with nocodazole (■), ~~taxol~~ the TAXOL™ drug (•) or colcemid (◆)12 hours after release from the cell cycle block or treated with nocodazole (X)14 hours after release."

Replace the paragraph at page 7, lines 23-25 with the following paragraph:

"Fig. 8D is a graph showing mitotic index of synchronized normal (primary) human osteoblasts in the absence (□) and presence of mitotic stress induced by ~~taxol~~ the TAXOL™ drug, T, (●) or nocodozole, N, (◆) 6 hours after release from the G1-S block."

Replace the paragraph at page 8, line 16 through page 9, line , with the following paragraph:

"As disclosed in the Examples 2 and 3 below, Chfr expression is ubiquitous in normal tissues. However, in three of eight human cancer cell lines, *chfr* mRNA and Chfr protein were undetectable. In a fourth human cancer cell line, a missense mutation was identified. The Chfr polypeptide is thereby inactivated due to lack of expression or by mutation in four out of eight examined human cancer cell lines. Normal primary cells, e.g., diploid fibroblasts, and tumor cell lines that express wild-type *chfr* exhibited delayed entry into metaphase (i.e., arrested in prophase) when exposed to an agent which disrupts microtubule function and induces mitotic stress. These agents, such as nocodazole, ~~taxol~~ the TAXOL™ drug and colcemid, inhibit centrosome separation. However, the tumor cell lines that have lost *chfr* function passed through prophase, entered metaphase without delay, and arrested in metaphase. Ectopic expression of wild-type *chfr* in these cells restored the cell cycle delay (e.g., prophase arrest) and increased the ability of the cells to survive mitotic stress. As discussed below, nocodazole inhibited centrosome separation, which normally occurs during prophase. Thus, cells that lack *chfr* function entered metaphase despite failure to separate the centrosomes. Such cells would be expected to have a high frequency of chromosome segregation errors and to survive mitotic stress less well than cells that retain *chfr* function. Thus, *chfr* defines a novel prophase to metaphase transition checkpoint that delays entry into metaphase in response to mitotic stress. A delay in metaphase entry in response to mitotic stress has not been previously described. When *chfr* is inactivated in human cancer cells, the inactivation contributes to aneuploidy and sensitivity to mitotic stress, e.g., such as that caused by agents that disrupt microtubule function or other chemotherapeutic agents."

Replace the paragraph at page 23, lines 19-24 , with the following paragraph:

"Also, as noted herein, pharmaceutical compositions of this invention may include a combination of compounds comprising a Chfr inhibitor and another chemotherapeutic agent, particularly an agent which disrupts microtubule function. Among such agents that disrupt microtubule function include nocodazole, ~~taxol~~ the TAXOL™ drug and colcemid. Other such agents known in the art, or that may be developed in the future should be useful in this context."

Replace the paragraphs at page 27, line 28 through page 28, line 15, with the following paragraphs:

"As the data presented in the following examples establish, inactivation of Chfr function or a lower level of expression thereof in human cancer has two effects. First, it predisposes the cell to aneuploidy, as cells that condense their chromosomes without having separated their centrosomes have difficulty forming an intact mitotic spindle. Second, it increases the sensitivity of cancer cells to mitotic stress. Thus, cancer cells lacking Chfr function would be sensitive to agents, such as nocodazole and ~~taxol~~ the TAXOL™ drug, that disrupt microtubule function, as demonstrated experimentally with the DLD1-neo and DLD1-*chfr* cells in the examples below

Thus, the present invention also provides a therapeutic method of retarding the growth of, or killing, tumor cells, by inhibiting expression of Chfr in cells which are tumor cells. Since the development of tumor cells occurs via a vast number of mechanisms, the tumor cells to be killed need not necessarily have arisen due to a lack of adequate expression of Chfr. Indeed, the method of killing tumor cells is likely to be more effective in cells in which Chfr is expressed, and which have developed into tumor cells via a Chfr-independent mechanism. In this instance, inhibition of Chfr expression results in a tumor cell which is more sensitive to mitotic stress and is therefore more sensitive to agents, such as nocodazole and ~~taxol~~ the TAXOL™ drug, that disrupt microtubule function."

Replace the paragraph at page 29, lines 1-11, with the following paragraph:

"This method may be performed by administering the pharmaceutical compositions described above via any suitable therapeutic route, and selection of such route is not a limitation of this invention. Similarly the appropriate dosage of such pharmaceutical compositions may be determined by a physician, based on typical characteristics such as the physical condition of the patient, the disease being treated, the use of other therapeutic compositions, etc. In one embodiment, the pharmaceutical compositions useful for practicing the therapeutic methods of the invention may be administered to deliver a dose of between 1 ng/kg/day and 100 mg/kg/day. The dosages of the agent which disrupts microtubule function, such as ~~taxol~~ the TAXOL™ drug, are known to those of skill in the art. This invention is therefore not limited by the dosage selection, which is within the skill of the art."

Replace the paragraph at page 35, line 10-19, with the following paragraph:

"All cancer cells were grown in DMEM supplemented with glutamine, penicillin, streptomycin and 10% fetal bovine serum (Life Sciences). Normal human epidermal keratinocytes and osteoblasts were grown in KGM2 and OGM media, respectively (Clonetics). The cells were examined either non-synchronized or synchronized. For synchronization, the cells were treated with 2 mM thymidine for 16 hours, then with 0.25 mM thymidine/deoxycytidine for 9 hours, and then with 0.5 μg/ml aphidicolin for 20-24 hours. The cells were washed three times with PBS between each step [Janss *et al.*, 1998, Exp. Cell Res., 243:29-38]. To induce mitotic stress, synchronized or non-synchronized cells were exposed to 0.5 μg/ml nocodazole, 5 μM ~~taxol~~ of the TAXOL™ drug or 0.5 μg/ml colcemid."

Replace the paragraph at page 36, line 27 through page 37, line 6, with the following paragraph:

"Cells synchronized by a sequential thymidine-aphidicolin block were either not exposed to mitotic stress or exposed to 0.5 μg/ml nocodazole or ~~taxol~~ the TAXOL™ drug for a 4 hour period starting 12 hours after aphidicolin release or release from the G1-S block. The short term response to mitotic stress was evaluated

by examining the cell cycle profile at the time of nocodazole removal, 24 or 48 hours later. At the indicated time points, the cells were recovered from the tissue culture plates with trypsin, fixed in 70% ethanol for 10 minutes and incubated with propidium iodide and DNase-free RNase (Roche) in PBS containing 1% fetal bovine serum and 2% Tween-20. The nuclear morphology of the cells was visualized by fluorescence microscopy. The DNA content of the cell population was determined by flow cytometry."

Replace the paragraphs at page 41, line 4 through page 42, line 2, with the following paragraph:

"To further strengthen the link between Chfr and the response to mitotic stress, experiments were performed to determine whether a dominant negative Chfr mutant would alter the behavior of cells, such as SAOS2, that express wild-type Chfr and have a low mitotic index in response to mitotic stress. Chfr-ΔFHA, a Chfr protein with deletion of residues 2-142 encompassing the FHA domain, was identified as a dominant negative mutant by studying its function in DLD 1 cells. Its effect on the response of SAOS2 cells to mitotic stress was studied by transiently-transfecting these cells with plasmids that express Chfr-ΔFHA or wild-type Chfr or no Chfr protein, together with a plasmid expressing GFP, as a marker. 36 hours later, mitotic stress was induced by exposure to ~~taxol~~ the TAXOL™ drug and the mitotic index was determined 8 to 16 hours later. Protein levels were determined by immunoblotting with an antibody that recognizes the N-terminal HA tag of the expressed Chfr proteins.

About 50% of the cells expressed GFP, but the variable level of expression made it difficult to define a threshold above which a cell would be considered GFP-positive. Thus, to avoid any bias, the mitotic index was calculated for the entire cell population. Expression of wild-type Chfr had no effect as compared to cells transfected with empty vector (Fig. 7B). However, Chfr-ΔFHA, whose level of expression was equivalent to that of wild-type Chfr led to a five-fold increase in the mitotic index at the 12, 14 and 16 hour timepoints, indicating a checkpoint defect. At the 8 hour timepoint, the mitotic index was low, similar to cells that lack Chfr (e.g.

DLD 1 and HCT 116), which begin to show a high mitotic index in response to mitotic stress 12-16 hours after addition of nocodazole or ~~taxol~~ the TAXOL™ drug. The effect of Chfr-ΔFHA in this assay was through dominant inhibition of endogenous wild-type Chfr based on an analysis of its function in transiently-transfected DLD1 cells, which lack endogenous Chfr. Chfr-ΔFHA had no effect on the mitotic index of DLD 1 cells exposed to mitotic stress, as compared to vector control, but inhibited the ability of wild-type Chfr to decrease the mitotic index. In the same assay, Chfr-M_{580} did not act as a dominant negative mutant (Fig. 7C)."

Replace the paragraph at page 42, lines 21-30, with the following paragraph:

"Similar results were obtained when mitotic stress was induced by colcemid or ~~taxol~~ the TAXOL™ drug, two other drugs that affect microtubule dynamics (Figs. 8A and 8B). Thus, Chfr regulates the prophase to metaphase transition in response to mitotic stress. Consistent with this role, the timing of induction of mitotic stress was critical for Chfr to delay entry in metaphase. Chfr did not affect cell cycle progression when nocodazole was added as the cells were entering metaphase 14 hours after aphidicolin release. In this case, both DLD1-neo and DLD1-chfr cells arrested in metaphase or entered metaphase with the same kinetics, as in the absence of mitotic stress (see, e.g., Figs.8A and 8B). Essentially identical results were obtained when U20S cells stably-transfected with plasmids expressing neo or wild-type chfr were examined. Furthermore, human primary epidermal keratinocytes and osteoblasts also exhibited a delay in metaphase entry in response to mitotic stress (Figs. 8C and 8D)."

Replace the paragraph at page 44, line 11 through 24, with the following paragraph:

"To further support the hypothesis that Chfr is a mitotic checkpoint, Chfr was examined to determine whether it affects cell viability in response to mitotic stress. Stably-transfected DLD1-neo and DLD1-chfr cells were synchronized by sequential thymidine-aphidicolin blocks and exposed to 0.5 μg/ml nocodazole or ~~taxol~~ the TAXOL™ drug for a 4 hour period starting 12 hours after aphidicolin release or release from the G1-S block. The short-term response of the cells to mitotic stress was

evaluated by examining cellular DNA content by flow cytometry and their nuclear morphology under the fluorescent microscope 48 hours later and the cell cycle profile at the time of nocodazole removal, 24 or 48 hours later. For microscopic examination, the cells were recovered from the tissue culture plates with trypsin, fixed in 70% ethanol for 10 minutes and incubated with propidium iodide and DNase-free RNase (Roche) in PBS containing 1% fetal bovine serum and 2% Tween-20. Further, after staining the cells with DAPI, the cells were inspected by fluorescence microscopy 64 hours after release from G1-S block."

Replace the paragraph at page 45, line 1 through 10, with the following paragraph:

"To the long term response of the cells to survive exposure to mitotic stress, synchronized DLD1-neo and DLD1-chfr cells were transiently exposed to nocodazole or ~~taxol~~ the TAXOL™ drug, as described above (e.g., exposed to nocodazole or ~~taxol~~ the TAXOL™ drug 12 hours after release from the G1-S block for a 4 hour period). At the time of nocodazole removal, the cells were replated at a density of 200 cells per 100 mm diameter tissue culture dish and then allowed to form colonies over a three-week period. DLD1-neo cells showed a decrease in the number of colony-forming units (CFUs) in response to mitotic stress, whereas for the DLD1-chfr cells the number of CFUs was unaffected by mitotic stress (Fig. 5). This provides additional evidence that Chfr expression leads to a low mitotic index in response to nocodazole."